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Strength of preference for nesting material as environmental enrichment for laboratory mice

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Abstract

The present paper describes two experiments in which preferences of laboratory mice for materials which could serve as cage enrichment were investigated. In the first experiment, presence of nesting material (paper towel or tissue) and the presence of a nest box (perforated metal or clear perspex box) were tested against each other. On average, the 47 mice tested spent significantly more time in the cage with the nesting material (more than 69% of their total time, whereas less than 25% of their time in the cage with the nest box). In the second experiment, the preferred nesting material (tissues) was placed in a cage with a grid floor (previously found to be avoided) and the nest box (perforated metal) was placed in another cage, connected to the first, with a solid floor covered with sawdust bedding material. In this experiment, 24 female mice were tested and on average they spent more than 67% of their time in the cage with the nesting material, despite the presence of a grid floor. Thus, it is concluded that providing a cage with nesting material (in addition to bedding) may be essential for the well-being of laboratory mice. © 1998 Elsevier Science B.V.

Keywords: Mouse; Enrichment; Preference strength; Nesting material

1. Introduction

Environments of laboratory animals have often been designed on the basis of economic and ergonomic aspects, with little or no consideration for animal welfare.

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Laboratory housing conditions can deprive animals of the possibility to perform a full repertoire of normal behaviour (Van de Weerd and Baumans, 1995). The inability to engage in species-specific behaviour may cause signs of suffering such as abnormal behaviour or pathology (Jensen and Toates, 1993). Environmental enrichment alters the environment by introducing materials or objects which are stimulating for the animals and which allow them to express more of their natural behavioural repertoire, thereby enhancing their well-being. Different animal species may have different enrichment requirements and when introducing enrichment to an animal's environment, it is very important to evaluate whether or not the animal responds to the enrichment. Preference tests can be used to determine some general principles about species-relevant properties of enrichment devices (Mench, 1994).

Previous studies on the preferences of laboratory mice for items which could serve as enrichment revealed clear preferences for a cage with nesting material or a nest box instead of a cage with only bedding material (Van de Weerd et al., 1997, 1998). Nesting material may have several functions. By building a nest, mice can regulate their temperature and avoid too much light or hide from aggressive cage mates. Nest boxes may provide a shelter or refuge because they give mice the opportunity to actively withdraw from frightening stimuli inside or outside their cage (Van de Weerd and Baumans, 1995).

The present paper describes two experiments in which these preferences are further investigated. The first experiment investigates whether mice prefer a nest box over nesting material or vice versa by testing the most preferred nesting material and the most preferred nest box from both previous studies against each other. In a second experiment, the importance or strength of the preference for nesting material was studied.

One general criticism of preference tests is that they only give information about the relative properties of the choices given, but do not indicate the importance an animal attaches to a preferred option. In order to interpret the results of preference tests and to be able to apply them to practical situations where an improvement in welfare is sought, the strength of the preferences should be established (Dawkins, 1983; Broom, 1988; Broom and Johnson, 1993; Duncan, 1992; Fraser, 1996). Where animals show that they are willing to work hard for the choices offered, it is reasonable to conclude that their welfare is improved by achieving that objective (Broom, 1988).

Several methods have been developed to measure the strength of preferences (also see Sherwin and Nicol, 1995), e.g., the instrumental or operant technique approach, where an animal has to learn to activate some mechanisms such as lever pressing or lifting a weighted door (Roper, 1973; Collier et al., 1990; Duncan, 1992; Manser et al., 1996) or the natural obstacle or obstructive techniques approach where an animal has to overcome a natural barrier such as a narrow gap or water (Duncan, 1992; Sherwin and Nicol, 1995). An animal may however, not always be able to learn an operant response (Duncan, 1992), it is therefore important that they associate the required activities with the goals to be reached and that the behaviour required for expressing the preference is reasonably natural for the type of reward (Fraser, 1996). Behaviours such as lever pressing or lifting a weight are not very natural for most animals.

In experiment 2 of this study, we have adopted the method of balancing one preference against another, as previously used by Van Rooijen (1980) with gilts,

Dawkins (1981, 1983) with hens and Blom et al. (1993) with mice. The testing variables (nesting material and nest box) were balanced against cage floor covering. Previous preference tests with the same strains of mice, showed that mice preferred bedding material and avoided wire mesh as floor covering (Blom et al., 1996). Thus, the preferred nesting material (Van de Weerd et al., 1997) was combined with the previously avoided grid floor and the preferred nest box (Van de Weerd et al., 1998) was combined with previously preferred bedding material. This approach will give an indication whether the mice are willing to accept a grid floor in order to use the nesting material or whether the combination of sawdust with the nest box is more attractive.

2. Animals, materials and methods

2.1. Experiment 1

2.1.1. Animals

Female and male mice of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU) were used, 12 animals per sex and strain group. Female and male mice were tested in separate groups. Testing of the females started when they were 13–14 weeks of age, whereas males were 30–31 weeks of age at the start of the experiment. One male BALB/cAnCrRyCpbRivU mouse died before the experiments started, leaving a total of 47 mice for the first experiment. All mice were familiar with the nesting material and nest box offered in the test series (either in previous preference test series or in their home cages).

2.1.2. Housing

The animals were housed (per strain and sex) in groups of six animals in a housing system consisting of two Macrolon type II cages (375 cm², UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system to allow the mice to get used to them. Both cages were supplied with food pellets *ad libitum* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad libitum* and sawdust bedding (Lignocel 3/4, Rettenmaier and Söhne, Ellwangen-Holzmühle, Germany). The animals were kept in conventional rooms with controlled photoperiod (12:12 light:dark, lights on at 07:00 h, approximately 200 lx at 1 m above the floor), temperature (20–22°C), relative humidity (50–60%) and ventilation (15 air changes h⁻¹). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lx at 1 m above the floor, in order to approach light intensities in standard animal rooms.

2.1.3. Preference test system

The preference test system used in this study has been validated and described in detail by Blom et al. (1992). In short, a multiple housing system was used consisting of two test cages (Macrolon type II) connected by nontransparent tubes (PVC, inner dimensions: 2.6 × 2.6 × 25 cm) to a central cage (15 × 15 × 18 cm, transparent perspex). The central cage was divided diagonally by a PVC sheet (19 × 17 cm). A total of

six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mice.

The test cages were supplied with a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photoelectrical devices in the passage tubes. The signals were sent to a computer which calculated dwelling times per cage (software: Gate-Watch, Metris System Engineering, Leiden, The Netherlands).

2.1.4. Behavioural observations

One of the six multiple housing systems was equipped with a videocamera system. Both test cages and the central one, were provided with a videocamera (Panasonic WV-1510). The cameras were connected with the photoelectrical devices, so the movements of the mice could be followed in the test system. The signals from the videocameras were sent to a time-lapse videorecorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment had red lights (approximately 5 lx at 1 m) to enable video recordings.

2.1.5. Procedure

Mice were introduced into the test system between 15:00 and 17:00 h and tested individually during 48 h. A group of six mice (of one sex and one strain) was tested simultaneously. The behaviour of one animal (selected randomly) was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). Food and water of each test cage were weighed before and after the experiment.

Per strain and sex group, the most preferred nesting material (Van de Weerd et al., 1997) was tested vs. the most preferred nest box (Van de Weerd et al., 1998), see Table 1. All test cages were supplied with 50 g of sawdust bedding (Lignocel 3/4).

Table 1
Materials tested in experiment 1

Animals	Nesting material (amount)	Nest box (8 × 10 × 6 cm)
<i>C57BL/6JcoU</i>		
males	Paper towel ^a (1 piece)	perforated metal
females	Kleenex [®] tissues ^b (2 pieces)	perforated metal
<i>BALB/cAnCrRyCpbRivU</i>		
males	Kleenex [®] tissues ^b (2 pieces)	clear perspex
females	Kleenex [®] tissues ^b (2 pieces)	perforated metal

^aCeltona, Cuijk, The Netherlands.

^bKimberly-Clark[®], EEC.

2.2. Experiment 2

2.2.1. Animals

Previous experiments with preferences for nesting materials (Van de Weerd et al., 1997) and nest boxes (Van de Weerd et al., 1998) revealed no major differences in preferences between the sexes of a strain, therefore in experiment 2 only female mice were used. The same female mice of experiment 1 were used in experiment 2, in order to explore their preferences further (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU, $N = 24$). At the start of experiment they were 16–17 weeks of age.

Housing conditions, test system and behavioural observations were similar as described for experiment 1 in Section 2.1.

2.2.2. Procedure

Testing procedure was similar to that in experiment 1 in Section 2.1.5. The perforated metal nest box of experiment 1 was offered in a test cage with 50 g of sawdust bedding and was tested against nesting material (Kleenex[®] tissues, 2 pieces). The nesting material was offered in a cage with an inserted wire grid floor (stainless steel wire, rod diameter 2 mm, mesh size $10 \times 10 \text{ mm}^2$). See Fig. 1.

2.2.3. Statistical analysis (experiments 1 and 2)

The dwelling data were analysed by distinguishing three time frames: the total dwelling times during the 48 h of the experiment, the dwelling times during 12 h of daylight (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronised with the periods of collected behavioural data (videotape recordings).

The method of statistical analysis used has been described by Blom et al. (1995). Briefly, per test series the dwelling time data (in seconds) were logarithmically



Fig. 1. Materials tested in experiment 2. Left: perforated metal nest box. Right: grid floor with two Kleenex[®] tissues.

transformed as they were not always normally distributed, and to increase independence of the data. For the same reason, central cage dwelling times were not included in the analysis. Data on food and water intake were not transformed, because they were normally distributed.

The data were analysed using paired *t*-tests to evaluate the influence of cage contents on choice behaviour and to detect possible differences in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was preset at $P < 0.05$.

2.2.4. Behavioural data

The behavioural data on videotape were viewed and analysed using a behavioural observation software package (The Observer, Version 2.0, Noldus Information Technology, The Netherlands). The time-lapse recorded tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom et al., 1992):

Sleeping in nest box or nesting material (sl-in)

Movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (e.g., turning) are not considered as an interruption.

Sleeping outside nest box or nesting material (sl-out)

Same as sleeping in, except the behaviour is performed outside the nest box or nesting material.

Grooming in nest box or nesting material (gr-in)

While sitting or standing, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside nest box or nesting material (gr-out)

Same as grooming in, except performed outside nest box or nesting material.

Manipulation (man)

Manipulation of the nesting material (shredding, fraying, dragging and nest building behaviour) or nest box (pushing, pulling, gnawing).

Ingestive behaviour (ingest)

Includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Exploration in nest box or nesting material (ex-in)

This includes all locomotion (movements), rearing (standing on hind feet, fore paws not touching the floor) and digging (pushing bedding material forwards or backwards with nose, fore paws or hind legs) performed in nest box or nesting material.

Exploration outside nest box or nesting material (ex-out)

Locomotion, rearing and digging performed outside nest box or nesting material.

Exploration on nest box (ex-on)

Locomotion and rearing on a nest box.

Climbing (clim)

Climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the passage tube or drinking nipple. While climbing or hanging, the hind legs or tail may touch the cage walls.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group ($N = 12$) were observed per test series. The results were used to describe the behaviour of the mice in the different test cages during a test series.

3. Results

3.1. Experiment 1

Fig. 2 illustrates the mean relative dwelling times per cage for experiment 1. Per strain and sex group, the mice spent on average significantly more time in the cage with

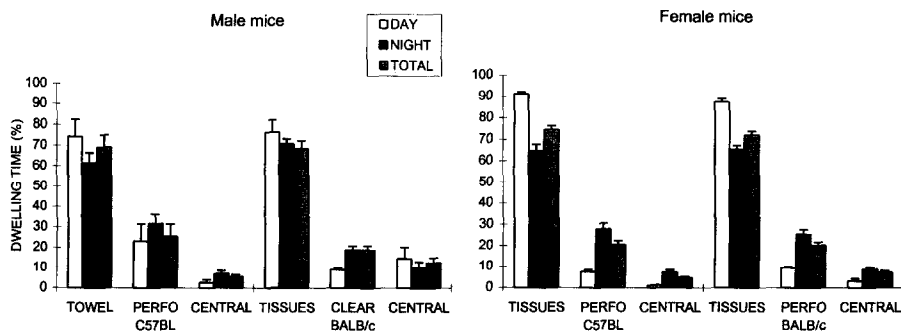


Fig. 2. Results of the preference test with a cage with nesting material (TOWEL = paper towel, TISSUES = Kleenex® tissues) and a cage with a nest box (PERFO = perforated metal, CLEAR = clear perspex), both connected to a central cage (CENTRAL). Mean relative dwelling times (and SEM) per cage for day (12 h), night (12 h) and total (48 h) period, for male and female mice of the C57BL/6JlcoU and BALB/cAnCrRyCpbRivU strains ($N = 47$).

the nesting material (day > 74%, night > 61%, total > 69% of the time; C57BL/6JlcoU male mice: paper towel, all $P < 0.05$; other groups: tissues, all $P < 0.001$) as compared to the cage with the nest box (day < 23%, night < 32%, total < 25% of the time) or central cage (day < 15%, night < 10%, total < 13% of the time). Significantly more food (60%, $P < 0.05$) was eaten by the BALB/cAnCrRyCpbRivU male mice in the cage with the (clear perspex) nest box than in the cage with the nesting material. The other groups did not eat significantly more food in either of the two cages. Female mice of both strains drank significantly more in the cage with the tissues as nesting material (> 61%, both strains $P < 0.05$).

3.2. Experiment 2

Fig. 3 illustrates the mean relative dwelling times per cage for experiment 2. Both groups of female mice spent on average significantly more time in the cage with the

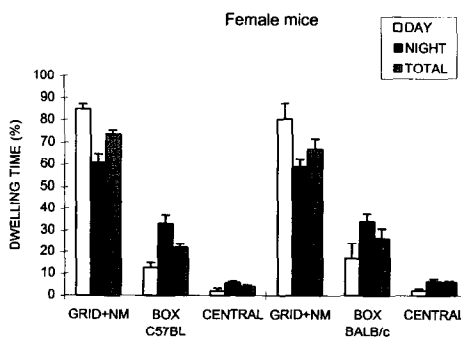


Fig. 3. Results of the preference test with a cage with nesting material (tissues) provided on a grid floor (GRID+NM) and a cage with a perforated metal nest box provided on sawdust bedding material (BOX), both connected to a central cage (CENTRAL). Mean relative dwelling times (and SEM) per cage for day (12 h), night (12 h) and total (48 h) period, for female mice of the C57BL/6JlcoU and BALB/cAnCrRyCpbRivU strains ($N = 24$).

nesting material on the grid floor (day > 81%, night > 59%, total > 67% of the time, all $P < 0.01$) as compared to the cage with the nest box on bedding (day < 17%, night < 34%, total < 27% of the time) or the central cage (day < 2%, night < 7%, total < 6% of the time). At least 59% of the water was taken up in the cage with the nesting material (tissues) on the grid floor (both strains $P < 0.05$), but no specific cage was chosen for food intake.

3.2.1. Behavioural data

Fig. 4 illustrates the distribution of active behaviour (i.e., excluding sleeping) for mice in both experiments. Only the night data are shown, because daytime data had similar patterns in both series. A lot of grooming outside the nest box (gr-out) was performed in both experiments, but grooming outside the nesting material was mostly seen in experiment 1 and not in experiment 2, where the nesting material was provided on the grid floor.

Fig. 5 shows the distribution of sleeping behaviour during the night in experiments 1 and 2. Most sleeping was performed in the nesting material (sl-in). In experiment 2 the

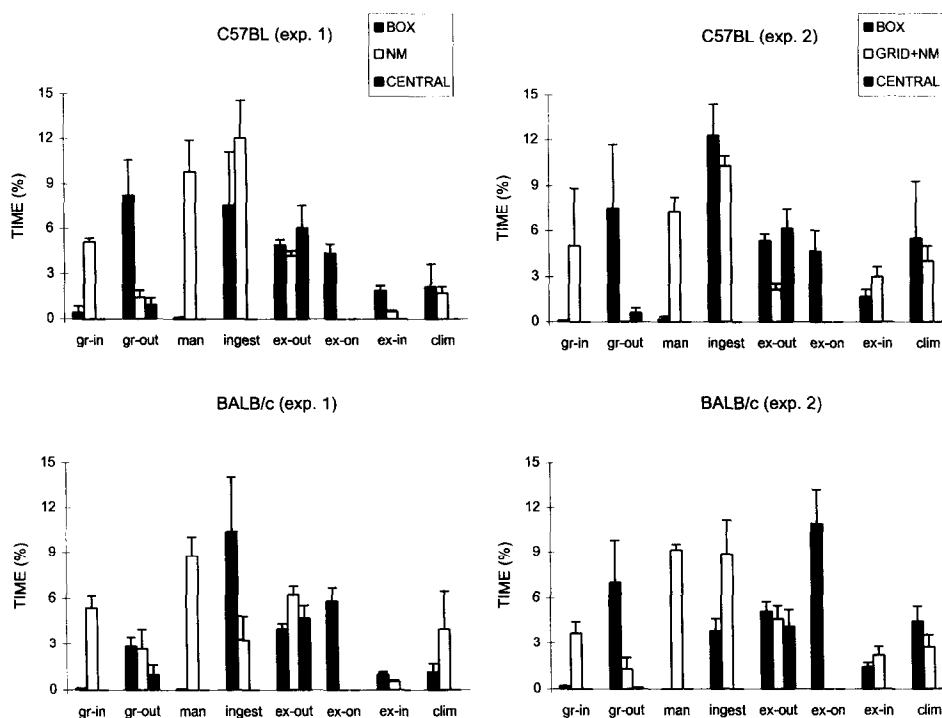


Fig. 4. Mean relative time (and SEM) spent on indicated active behaviour during the night time period (12 h). Left: experiment 1 of Section 2.1, preference tests with nesting material (NM) vs. a nest box (BOX) with male and female mice of the C57BL/6JcoU and BALB/cAnCrRyCpbRivU strains, $N = 8$. Right: experiment 2 of Section 2.2, preference tests with nesting material on a grid floor (GRID+NM) vs. a nest box on bedding material (BOX) with female mice of the C57BL/6JcoU and BALB/cAnCrRyCpbRivU strains, $N = 4$. CENTRAL = central cage. See Section 2.2.4 for explanation of abbreviations.

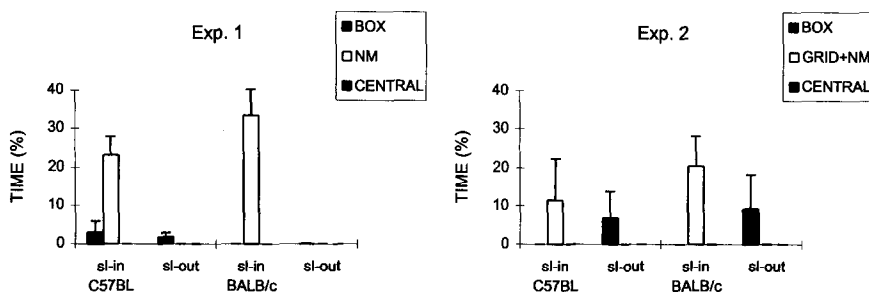


Fig. 5. Mean relative time (and SEM) spent on sleeping behaviour during the night time period (12 h). Left: experiment 1 of Section 2.1, preference tests with nesting material (NM) vs. a nest box (BOX) with male and female mice of the C57BL/6JlcoU and BALB/cAnCrRyCpbRivU strains, $N=8$. Right: experiment 2 of Section 2.2, preference tests with nesting material on a grid floor (GRID+NM) vs. a nest box on bedding material (BOX) with female mice of the C57BL/6JlcoU and BALB/cAnCrRyCpbRivU strains, $N=4$. CENTRAL = central cage. See Section 2.2.4 for explanation of abbreviations.

mice slept more in the cage with the nest box than in experiment 1. They slept outside the nest box (sl-out) about 10% of the time in experiment 2, which was much greater than during experiment 1, when the nesting material was provided on the bedding.

4. Discussion

All groups of mice showed a clear preference for the nesting material in experiment 1, with 60–90% of the time spent in the cage with the nesting material. Cages with nest boxes were mainly visited during the night, when the mice were active and explored the test system (see Fig. 4). In previous preference tests with different types of nest boxes, mice preferred a cage with a nest box to one with no nest box (Van de Weerd et al., 1998), but in the present experiment the nesting material appeared to be more attractive. This was also shown clearly in experiment 2, where again, all mice had a strong preference for the cage with the nesting material even when a grid floor was present. Previous studies have shown that rodents avoid grid floors when alternatives are offered (Arnold and Estep, 1994; Manser et al., 1995, 1996; Blom et al., 1996, in press; Van de Weerd et al., 1996).

In previous preference tests with nesting material, approximately half the number of (naive) mice made a combination of the most preferred nesting materials by dragging them from one cage to another (Van de Weerd et al., 1997). In the present study, this behaviour was not seen. In both experiments, all mice spent most time in the cage with the nesting material and even in experiment 2, the mice did not drag the nesting material to the cage with the nest box to combine both commodities or at least lie in the bedding. It can be argued that they accepted the grid floor in order to rest in the nesting material, as the nesting material masks the structure of a grid floor. Fig. 6 shows the type of nests the mice made on the grid floors, which have the same shape as nests constructed on bedding.



Fig. 6. Example of a nest built on the grid floor (experiment 2 of Section 2.2).

These results imply that nesting materials are much more attractive for mice than nest boxes. In natural settings, nest boxes may be used by rodents as a feeding post, as a storage for food, for the construction of a nest or for the bearing and raising of offspring as described by Ryszkowski and Truszkowski (1970). They may also offer an opportunity to hide from predators. In the laboratory the function of a nest box is more restricted, the main function probably is to offer a shelter against overexposure to light or to avoid aggressive cage mates (Van de Weerd and Baumans, 1995). Nesting material has similar functions, but differs from nest boxes in that it can be manipulated to build a nest and by doing this, the mice are able to structure their environment (Van de Weerd et al., 1997). Another main function of a nest is to shelter animals from variations in environmental temperatures (Brain and Rajendram, 1986). Both males and females will build a nest when offered nesting materials and there is a strong genetic influence on nest-building behaviour (Lisk et al., 1969; Lee, 1972, 1973; Lynch and Hegmann, 1972; Brain and Rajendram, 1986).

Several studies have shown that mice are willing to work in order to get nesting material, e.g., when nesting material is put on the cage lid, they start pulling it into the cage (Lisk et al., 1969; Lynch and Hegmann, 1972; Wolfe and Barnett, 1977). Roper (1973) showed that mice can be trained to press a key in order to obtain nesting material (paper strips). The paper acted as a reinforcer for this response. Collier et al. (1990) described a similar experiment with rats, which were motivated to press a response bar often in order to reach a nest, although they were willing to press more in order to reach food or water. This phenomenon has also been described in other studies, which compared the demand for certain behavioural activities with the demand for food or water (Dawkins, 1983; Matthews, 1994; also see discussion in Roper, 1973). In general, animals are willing to work harder for food or water than for other commodities. This is not surprising, because animals will almost always be highly motivated to gain access to food and water because this is an essential need for survival (Matthews, 1994).

Measurement of the motivation of an animal to obtain a resource will be dependent on the alternatives offered, the elasticity for the demand will be greater when it is more substitutable for a commodity concurrently available (Lea and Roper, 1977; Barnett and Hocking, 1981). Sherwin and Nicol (1995) combined food-searching behaviour with the occurrence of natural obstacles (air stream, water or a narrow gap). The willingness to overcome these obstacles to obtain food was used as a yardstick. Dawkins (1981) examined the priorities hens gave to two features of their environment, namely size and flooring of a cage. A comparable approach was used in experiment 2 of the present study, in which commodities addressing related behavioural activities were compared, i.e., type of cage flooring (bedding or grid) combined with materials offering shelter (nest box or nesting material). Mice preferred the nesting material, although during the night some mice slept in the bedding of the cage with the nest box, but not inside the nest box (see Fig. 5). This practical approach allows for the comparison of various environmental aspects and may directly lead to designs for better housing conditions.

Laboratory environments are barren and often poorly structured and contain few features that can be manipulated or changed by the animal's behaviour. This makes it difficult for animals to adopt a behavioural response that reduces the effect of aversive stimuli (coping) in stressful situations (Wechsler, 1995). By providing nesting material, mice are able to structure their environment by manipulation of the nesting material and this gives them more control over their living conditions. More control may enhance their well-being (Beaver, 1989; Chamove, 1989; Van de Weerd and Baumans, 1995). Nesting material also allows mice to perform species-specific nest-building behaviour. The inability to engage in species-specific behaviour may cause signs of suffering and the mere possibility to perform certain behaviours may decrease the physiological effect of stressful situations (Jensen and Toates, 1993). Species-specific behaviour has evolved from continuous adaptations to the natural environment. Despite generations of domestication of mice in the laboratory, adaptive behavioural strategies such as burrowing are still present in laboratory strains and do not appear to be different from wild mice (Adams and Boice, 1981). Nest building is related to burrowing activities (Brain and Rajendram, 1986) and can be seen as an active strategy of a mouse to control its environment (Sluyter et al., 1995).

5. Conclusion

Housing systems should be designed to allow animals to perform effective coping behaviour when confronted with aversive stimuli, in order to prevent poor welfare (Wechsler, 1995). When housing systems cannot be altered immediately, the provision of environmental enrichment such as nesting materials may be a relatively easy, short-term solution to enhance well-being. Natural selection, domestication and experience have shaped decision making in animals in such a way that the resultant behaviour is optimally adapted to the current environmental circumstances. In general, this will enhance biological fitness and promote welfare (McFarland, 1977; Fraser, 1996). It is therefore reasonable to conclude that animal welfare is improved by achieving the objective the animal is willing to work for, and that reaching this objective is

experienced as positive (Van Rooijen, 1983/1984; Broom, 1988). Mice in this study were highly motivated to lie in nesting material, even when presented on a grid floor. Thus we may conclude that nesting material has a positive effect on their well-being.

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